

TITLE: DELETIONS IN ARTERIVIRUS

REPLICONS

Inventor: Monique Helene Verheije

Docket No.: 2183-6217US

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Constructs						Deletion (nucleotides)	PABV number	M-expression	N-expression
5'UTR- ORF1ab	ORF2	ORF3	ORF4	ORF5	ORF6	ORF7	3'UTR		
##	—	—	—	—	—	—	AAA	437	+
##	—	—	—	—	—	—	AAA	594	+
##	—	—	—	—	—	—	AAA	Δ 11788-14139	-
##	—	—	—	—	—	—	AAA	Δ 14585-14984	-
##	—	—	—	—	—	—	AAA	521	1)
##	—	—	—	—	—	—	AAA	Δ 11788-14584	-
##	—	—	—	—	—	—	AAA	664	+
##	—	—	—	—	—	—	AAA	Δ 14985-15111	-
##	—	—	—	—	—	—	AAA	668	1)

1) Identical results were obtained in IPMA using MAbs against GP₃ and GP₄

Fig. 1A

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Constructs	Deletion (nucleotides)	PABV number	M- expression
5'UTR- ORF6 <i>Hpa</i> I ATG ORF7 <i>Pst</i> I TAA 3'UTR AAA		437	+ ¹⁾
AAA	Δ 14588-14936	605	-
AAA	Δ 14588-14885	604	-
AAA	Δ 14588-14786	603	-
AAA	Δ 14588-14687	602	-
AAA	Δ 14588-14642	624	+
AAA	Δ 14599-14642	625	+
AAA	Δ 14588-14600	626	+ ¹⁾
AAA	Δ 14938-14980	638	+ ¹⁾
AAA	Δ 14887-14980	637	+
AAA	Δ 14788-14980	636	+
AAA	Δ 14686-14980	635	+
AAA	Δ 14643-14686	631	-
AAA	Δ 14643-14676	632	-
AAA	Δ 14643-14664	633	-
AAA	Δ 14643-14652	634	+
AAA	Δ 14653-14686	696	-
AAA	rescue of 696	730	+ ¹⁾

¹⁾ Identical results were obtained in IPMA using MAb 122.17 against N

Fig. 1B

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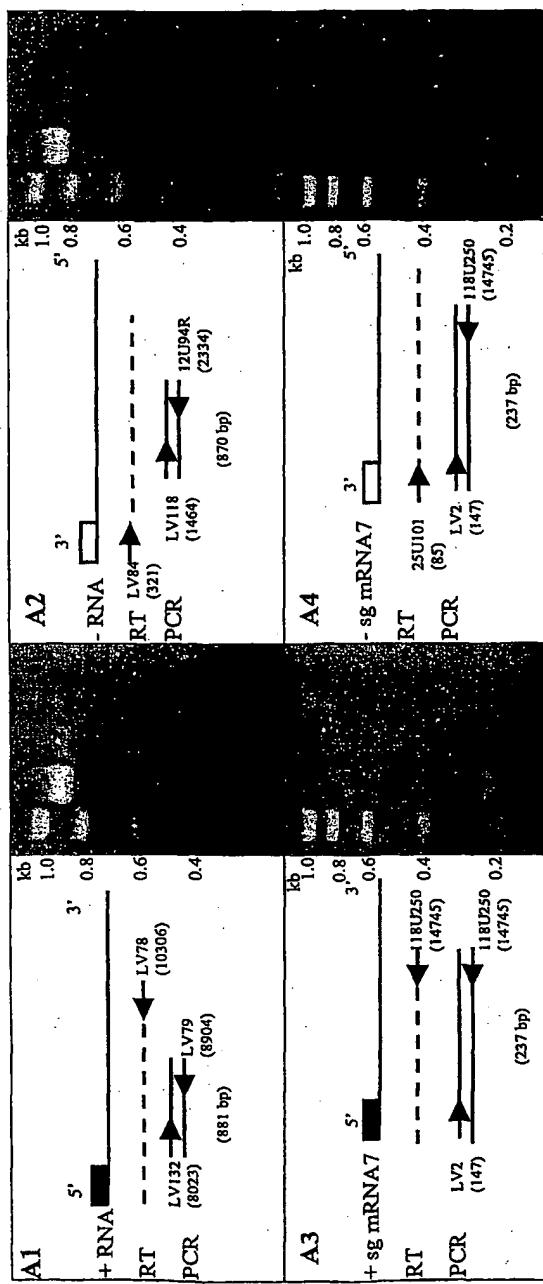
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Fig. 2



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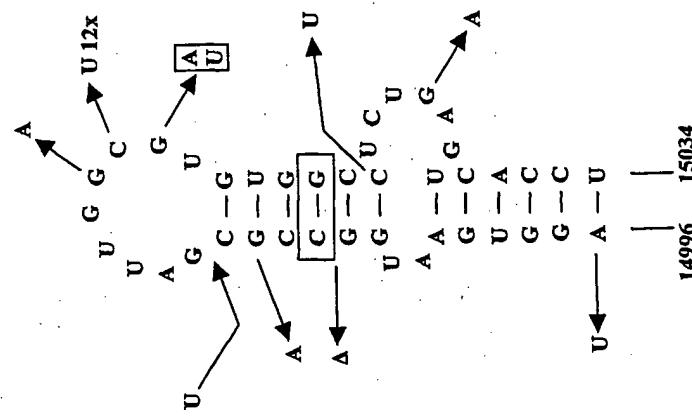


Fig. 3B

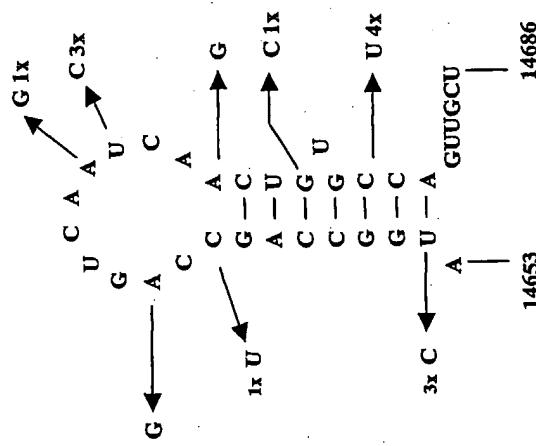


Fig. 3A

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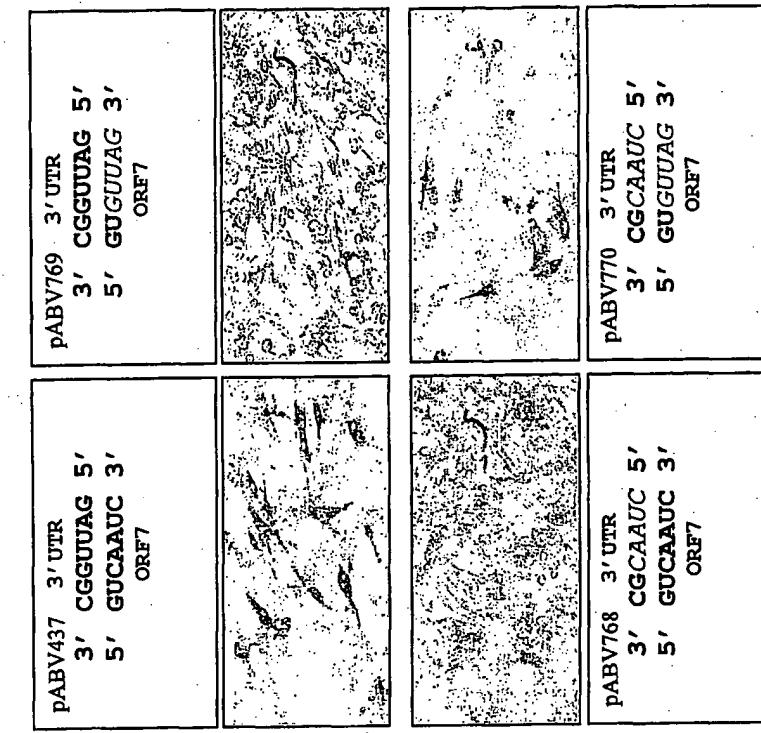
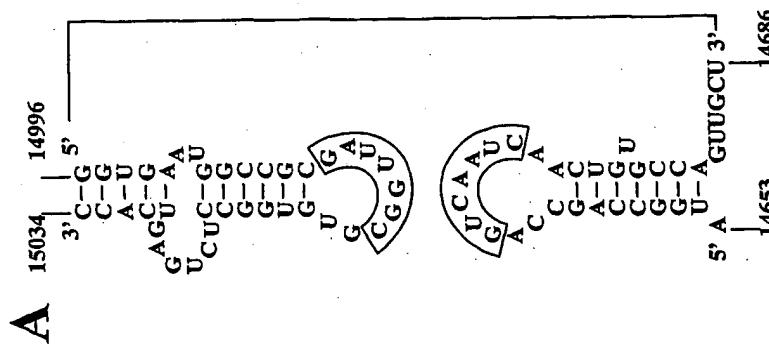


Fig. 4



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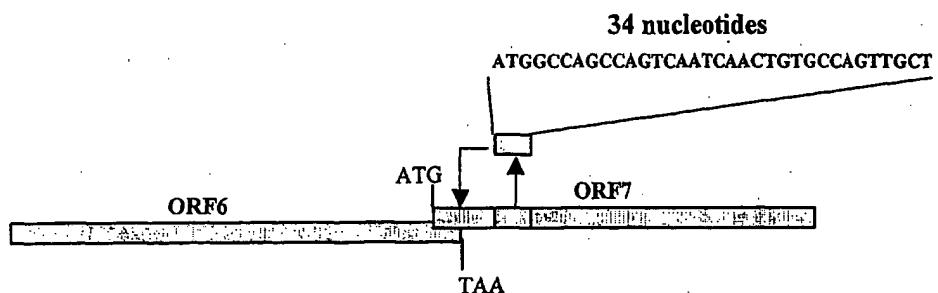


Fig. 5

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LV	MAGKNOQQKKKSTAPMNGQQPNOLCOLLGAMIKSQRQ---OPRGQAKKKKKPEKPHPEPLAEDDIRHH	67
VVR2332	MPNNNGKQQKRK---KGDGQPNOLCOMLGKLLAQOQNOSRGKGGKNNKPEKPHPEPLATEDDVRRHH	66

LV	TTAACAGTCA	GGTCAATGGCCGGATTGGCG	32
VR2332	TGGCTGCAATTCTTGAGGCTATCTCACTGTTGAATTGGAAAGATGTTGTCAGTCA	TTGCTGCAATTCTTGAGGCTATCTCACTGTTGAATTGGAAAGATGTTGTCAGTCA	70

LV	: TGTGGCCTGAGTCACTTCAATTAGGGATCACATGGGGTCATACTTATATCAGGGAGAACCT	: 102
VR2332	: TTGTGGCCTAAGTCACTTCAATTAGGGACCGTGTGGGTGAGATTATT-GCCGAGACCTT	: 139

LV : GTGACCGAAATTAAAAAA: 122
 VR2332 : GGGCCCGAAATTAAAAAA: 159
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Constructs	Deletion (nucleotides / amino acids)	Plasmid number	M- expression	N- expression	Virus production
A B C D			+	+	+
ORF7 3'UTR	wild type	437	+	+	+
##	Δ14975-14980 / Δ 2	639	+	+	+
##	Δ14969-14980 / Δ 4	694	+	+	+
##	Δ14966-14980/ Δ 5	745	+	+	+
##	Δ14963-14980/ Δ 6	746	+	+	+
##	Δ14960-14980/ Δ 7	747	+	+	+
##	Δ14957-14980/ Δ 8	748	+	+	+
##	Δ14954-14980/ Δ 9	695	+	+	+
##	Δ14989-14995	693	-	-	-
##	Δ14989-15020	729	-	-	-

Fig. 7

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Growth curves of PRRSV deletion mutants

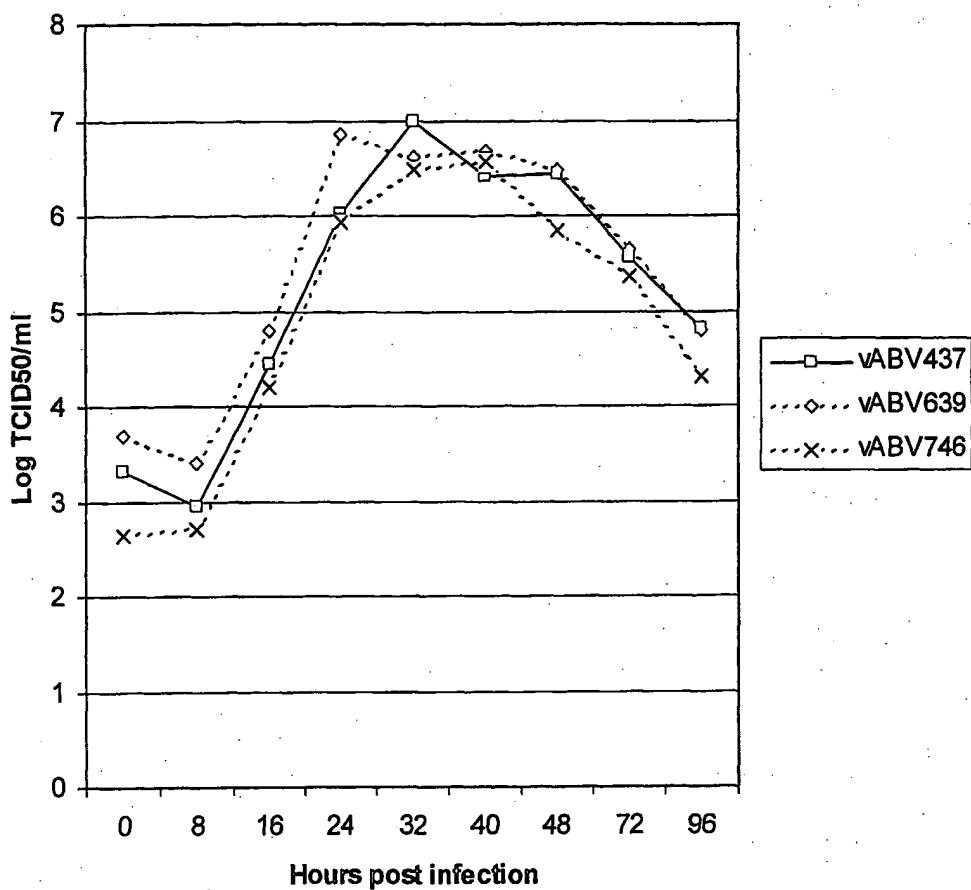
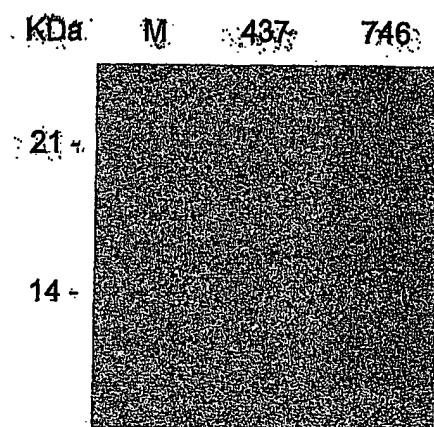


Fig. 8

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Fig. 9

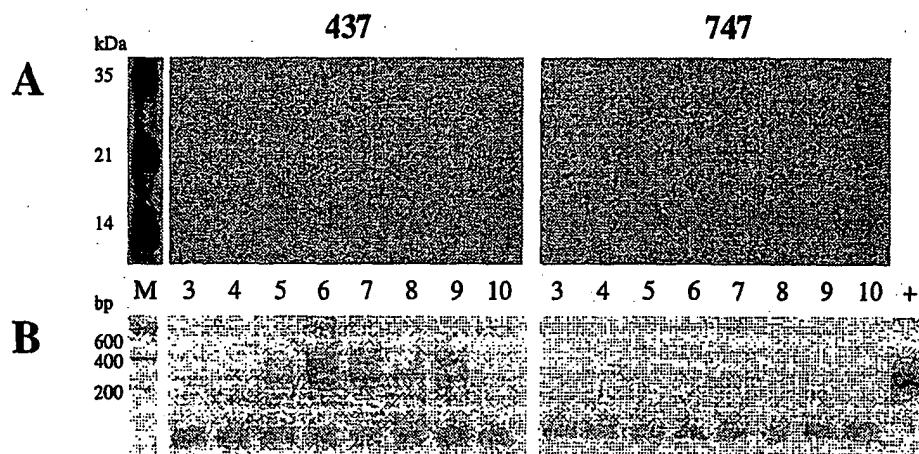


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Fig. 10



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TABLE 1: Sequences of the primers used to introduce deletions by PCR, and primers used to sequence the introduced mutations.

Primer	Sequence of the primer ^a	Orientation	Purpose (pABV)	Location
119R218R	5' ATGACATCCGGCACCC 3'	+	Sequencing	14782
LY20	5' CCTGATTAAAAGCTTGACCCC 3'	-	Sequencing	15066
LY75	5' TCTAGGAATTCTAGACGATCG 3'	-	XbaI -site	15088
LY155	5' ACGTGCCTTAACCTCGTCAAGTATGGCGGTAAAAACCAAGAGCCAGA 3'	+	HpaI-site	14582
LY204	5' ACGTGCCTTAACCTGACTGGGGATGTAGA 3'	-		639
LY213	5' TGCAAGTTAAATTAAAGGTGAATGGCCCGCGA 3'	+		693
LY 214	5' GACTGTTAAATTAAACTGGGGATGTA 3'	-		694
LY215	5' GACTGTTAAATTAAAGTCACGGGAATC 3'	-		695
LY239	5' TGCAAGTTAAATTAAAGCTCTGAGTCA 3'	+		729
LY263	5' GACTGTTAAATTAAAGCTGAGTGA 3'	-		745
LY264	5' GACTGTTAAATTAAAGTAGAAGTC 3'	-		746
LY265	5' GACTGTTAAATTAAAGTAGAAGTCACG 3'	-		747
LY266	5' GACTGTTAAATTAAAGAGTCACGCGA 3'	-		748

^aThe restriction sites are underlined.

Fig. 11

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Fig. 12

TABLE 1: Sequences of the primers used to introduce deletions by PCR, primers used to sequence the introduced mutations, and primers used for the strand-specific RT-PCR

Primer	Sequence of the primer ^a	Orien-tation	Pur-pose (pABV)	Loca-tion
118U250	5' CAGCCAGGGAAAAAATCGGGC 3'	-	Sequencing / Strand-sp. PCR	14745
12U94R	5' CACCTGTAACCTGCTCATTTG 3'	-	Strand-sp. PCR	2334
25U101	5' GTTCTAGCCAAACAGGTATC 3'	+	Strand-sp. RT	85
LV2	5' AGGGAAAGGATCCACGGAGTAT 3'	+	Strand-sp. PCR	147
LV17	5' CCCTTGACGAGCTTCGGC 3'	+	Sequencing	14045
LV20	5' CCTGATTAAAGCTTGACCCCC 3'	+	PCR-XbaI-site	15066
LV75	5' TCTAGGAATTCTAGACGATTCG 3'	-	RT	15088
LV76	5' TCTAGGAATTCTAGACATCGT40 3'	-	Strand-sp. RT	15088
LV78	5' CCCTGGGATGAATTTAGCTATGG 3'	-	Strand-sp. PCR	10306
LV79	5' GACAAGATCAAGATTAATACC 3'	-	Strand-sp. RT	8904
LV84	5' AGACCTCAGGACACTGACC 3'	+	Strand-sp. RT	321
LV112	5' CCATTCACTCTACTCTCCACCG 3'	+	PCR-PacI-site	14981
LV118	5' TTACCACTACTCTCCATATGTTC 3'	+	Strand-sp. PCR	1464
LV132	5' CCTACTGTGCCCTATATGTTC 3'	+	Strand-sp. PCR	8023
LV151	5' ACCAGAGCCAGAAGAAAAAGAACAGTACAGCTGGTCCAATGAT 3'	+	PCR (631)	14611
LV152	5' ACCAGAGCCAGAAGAAAAAGAACAGTACAGCTGGCAGATGG 3'	+	PCR (632)	14611
LV153	5' ACCAGAGCCAGAAGAAAAAGAACAGTACAGCTTAATCAACTGT 3'	+	PCR (633)	14611
LV154	5' ACCAGAGCCAGAAGAAAAAGAACAGTACAGCTATGCCAGCAG 3'	+	PCR (634)	14611
LV155	5' ACCTGCGTTAACCTCGTCAAGTATGCCGGTAAAAACCAGAACCCAGA 3'	+	HpaII-site PCR	14582
LV188	5' ACCTGCGTTAACCTCGTCAAGTATGCCGGTAAAAACCAGAACCCAGA 3'	+	PCR (602)	14582
LV189	5' ACCTGCGTTAACCTCGTCAAGTATGCCGGAACACCTCACCA 3'	+	PCR (603)	14582
LV190	5' ACCTGCGTTAACCTCGTCAAGTATGCCGGAACACCTCACCA 3'	+	PCR (604)	14582
LV191	5' ACCTGCGTTAACCTCGTCAAGTATGCCGGAACACCTCACCA 3'	+	PCR (605)	14582
LV195	5' ACCTGCGTTAACCTCGTCAAGTATGCCGGAACACCTCACCA 3'	+	PCR (624)	14582
LV196	5' GGACTGCGTTAACCTCGTCAAGTATGCCGGAACACCTCACCA 3'	+	PCR (625)	14582
LV197	5' ACCTGCGTTAACCTCGTCAAGTATGCCGGAACACCTCACCA 3'	+	PCR (626)	14582
LV198	5' GCTCGTGTCTAGCCTTAAGCATCACATACAC 3'	+	HpaII-site PCR	14140
LV200	5' ACGTGCTTAATTAACCCAGAACCTGACAGTTG 3'	-	PCR (635)	14981
LV201	5' ACGTGCTTAATTAATGTCATCTTCAGGCCAG 3'	-	PCR (636)	14981
LV202	5' ACGTGCTTAATTAACCGTGGATGAAAGGACGC 3'	-	PCR (637)	14981
LV203	5' ACGTGCTTAATTAACCGACTGTATGCCAACCGG 3'	-	PCR (638)	14981
LV204	5' ACGTGCTTAATTAACCGACTGTATGCCAACCGG 3'	-	PCR (639)	14981
LV216	5' ACCAGAGCCAGAAGAAAAAGAACAGTACAGCTGGATGGGGAG GGTGCAATGAT 3'	+	PCR (696)	14611
LV268	5' ACCAGAGCCAGAAGAAAAAGAACAGTACAGCTGGATGGGGAG 3'	+	PCR (769)	14611
LV269	5' CTCGATGGGAAATGCCAGGCCAGTGTGGCTTAACGCGGTGGCCTC 3' +	+	PCR (769)	14641
LV270	5' TGCAAGTTAAACACTAGGTGAATGCCCTAAGCGTGGCCTC 3' +	+	PCR (768)	14981

^aThe restriction sites are underlined.